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Original Article

Comparing the effects of swimming and running wheel on clinical symptoms and myelin basic protein in mice with experimental autoimmune encephalomyelitis

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Abstract

Background and aims: The present investigation examines the impact of aerobic exercises, especially swimming and running wheel exercises, on the clinical manifestations and myelin basic protein (MBP) levels in mice with experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS). MBP is critical for the formation of myelin sheaths within the nervous system, and its deterioration is linked to the pathogenesis of MS.

Methods: The sample of the study consisted of 96 female C57BL/6 mice. After the EAE induction, the mice were divided into 8 groups. The animals performed two exercise protocols. Then, the brain tissue was isolated, and levels of the mentioned variables were measured via the ELISA method using specific kits. The data were analyzed statistically using one-way ANOVA.

Results: Results showed that lesion scores in both exercise protocols were lower in EAE mice than those in the control groups ($P=0.001$); in other words, swimming and receiving interferon-beta-1 reduced MBP degradation, but the decrease was not significant ($P=0.09$). Additionally, running wheels reduced MBP degradation, which was statistically significant ($P=0.001$).

Conclusion: Results suggested that using a voluntary running wheel might be a more effective exercise program than swimming in preventing MBP degradation in the brain tissues of mice with EAE.

Keywords: Multiple sclerosis, Training, Interferon, Symptoms

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Introduction

The pathophysiological mechanisms of multiple sclerosis (MS) are complex (1-3). It has been shown that axon inflammation and degeneration may be involved in the pathogenesis of MS (4). Research has indicated that experimental autoimmune encephalomyelitis (EAE), an animal model for MS, seems ideal for investigating the influence of physical activity on the underlying mechanisms of MS (5). Regular exercise can help suppress pathogenic infections by increasing antigen-specific immune responses (6,7). Regular aquatic exercise such as swimming can enhance muscle strength and amplify the anti-inflammatory responses of the body while providing neuroprotective benefits (8). Experimental investigations utilizing animal samples have demonstrated that engaging in physical activities, such as using a running wheel, can facilitate pathophysiological recovery and cognitive enhancement following cerebral trauma. Research findings indicate that forced physical activity positively influences neural functionality and may improve symptoms associated with neurological disorders (9). For example, 6 weeks of aerobic exercise on a treadmill neutralized the deleterious effects of EAE on blood-brain barrier integrity and consequent neuronal apoptosis (10). The progressive stage of MS is characterized by neurological impairment, demyelination, and axonal damage, which can result in physical disability and cognitive impairments (11).

The entry of inflammatory factors into the central nervous system (CNS) is triggered by a complex interplay between β-cells, T lymphocytes, macrophages, and many signaling molecules such as cytokines and chemokines, along with various inflammatory pathogens. This multifaceted inflammatory response culminates in the destruction of myelin sheaths, which is followed by the demise of oligodendrocytes, the cells responsible for myelin production, and the subsequent breakdown of myelin proteins, including myelin essential protein (MBP) and proteolipid protein (PLP) (12,13). Investigations into the biophysical and biological characteristics of MBP have advanced our understanding of its function as an essential element in the structural and communicative nexus between oligodendrocytes and myelin. Although the absence of myelin-associated glycoprotein (MAG), PLP, or 2',3'-cyclic nucleotide 3'-phosphodiesterase (CNP)

exhibits minimal immediate impact on the formation of myelin, the progressive adverse effects on axonal integrity are evident. Contrastingly, mice deficient in MBP fail to synthesize myelin altogether, underscoring the critical role of MBP in the pathophysiology of MS (14). In this model, the MPB level is studied to determine the effect of aerobic exercise on preventing and improving demyelination processes (5).

Given the limited evidence of the efficacy of new drugs and their high cost, non-pharmacological interventions such as exercise have emerged as promising alternatives (15). Numerous clinical studies have explored the effects of exercise on molecular, histopathological, and behavioral abnormalities in MS patients and animal models (1). Accordingly, our study delved into the potential of regular exercise and neuroprotection enhancement, demonstrating a delayed onset of EAE, decreased clinical scores, and inflammatory responses associated with reduced post-EAE demyelination (16). The genetic predisposition to MS, particularly the role of the MBP gene was also considered in a previous study, where the mortality rate of mice treated with MBP showed 20%-30% improvement compared to control groups (17). A recent study has hinted at the benefits of regular physical exercise in reducing neural inflammation and degeneration (18); however, it did not explore the effects and types of exercise environments. Our unique approach, focusing on the mediating role of MBP in the relationship between exercise and the body, particularly its role in remyelination, aims to answer the research question: Do four weeks of aerobic swimming and vulnerary running wheel affect clinical symptoms and MBP levels in the hippocampus of mice with EAE? By studying environmental conditions and types of physical activities, we hope to offer new and promising strategies for treating MS.

Materials and Methods

Samples and study design

In this experimental investigation, a total of 96 female C57BL/6 mice, aged between 10 and 12 weeks with an average weight of 25 ± 2 g, were procured from the Pasteur Institute of Iran (Table 1). These mice were housed under controlled environmental conditions, including a 12-hour light/dark cycle and an ambient temperature of 23 ± 1 °C. The food was supplied by Behparvar Industrial Company, and water was made available ad libitum (19-20). The experimental protocols employed in this study received approval from the Research Ethics Committee of Chamran University in Ahvaz, ensuring adherence to ethical standards in treating the animal subjects.

Experimental groups

The mice were randomly divided into eight groups of 6, and the groups were named as follows:

(1) control group, (2) healthy exercise group, (3) EAE + saline injection group, (4) EAE control group, (5) EAE + interferon group, (6) exercise environment + saline injection group, (7) EAE + exercise group, and (8) EAE + exercise + interferon group.

Table 1. Initial weights of C57BL/6 mice in different groups

Groups	Running wheel group	Swimming group
Healthy group	25 ± 1	26 ± 1
EAE control	23 ± 1	25 ± 1
Healthy exercise group	26 ± 1	26 ± 1
EAE + exercise	24 ± 1	24 ± 1
EAE + saline injection	24 ± 1	24 ± 1
EAE + interferon	27 ± 1	27 ± 1
EAE + exercise + saline injection	23 ± 1	24 ± 1
EAE + exercise + interferon	25 ± 1	25 ± 1

Mice with EAE

At the Salari Institute for Cognitive and Behavioral Disorders, a protocol was implemented to induce EAE in murine subjects. The initiation of EAE involved a two-step process. First, the mice were sedated with an intraperitoneal injection of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg). Subsequently, an emulsion containing 300 µg of MOG peptide (35-55) diluted in 100 µL of phosphate-buffered saline (PBS) was combined with 500 µg of Mycobacterium tuberculosis extract in 100 µL of complete Freund's adjuvant. This mixture was administered subcutaneously on both flanks of the subjects.

To augment the inflammatory response and enhance CNS infiltration, 300 ng of pertussis toxin was administered intraperitoneally immediately after the initial injection and again after 48 hours. This toxin increases the permeability of the blood-brain barrier, thereby facilitating the entry of inflammatory cells into the CNS. The progression and intensity of EAE were meticulously monitored and graded using a scale ranging from 0, indicating the absence of clinical signs, to 5, which indicated either severe paralysis or death. Intermediate scores were assigned as follows: 0.5 for a loss of tail tone, 1 for complete tail paralysis, 1.5 for tail paralysis coupled with mild hind limb weakness, 2 for pronounced hind limb weakness, 2.5 for paralysis affecting one side, 3 for complete paralysis of hind limbs, 3.5 including forelimb weakness, and 4 representing paralysis extending to all four limbs.

Aerobic swimming

Based on the experimental design delineated by Bernard et al, an aquatic exercise program was initiated 9 days after administering experimental EAE. The protocol entailed immersing the mice in water while maintaining a temperature of approximately 31 °C for half an hour each day over five days per week, continuing for 4 weeks. The initial phase of the program, spanning 4 days, was dedicated to acclimatizing the animals to the aquatic environment and the mechanics of swimming. On

the fifth day, the mice were subjected to the structured exercise protocol outlined by Bernardes et al, which included progressively increasing resistive loads attached to their tails, starting at 2% of their body mass (21).

The endurance of the mice was tested by submerging them until signs of fatigue were evident, ensuring that immersion did not exceed 3 minutes. The protocol dictated incremental increases in resistive load by 2%, culminating in a determination that the maximum burden tolerable by the mice was 7% of their body mass. Consequently, the inaugural swimming session imposed a load equivalent to 60% of their maximal capacity, translating to 4.2% of their body weight. The mice were subjected to weekly weigh-ins to adjust the resistive loads according to their updated weights. However, observations indicated that the weights of the mice remained stable throughout the experiment, obviating the need for any alterations in the applied loads (21).

To simulate the atmospheric conditions of the swimming apparatus and ensure consistent humidity levels for the control group, these mice were placed on an elevated platform within the same vicinity as the experimental group's swimming chamber. Post-exercise recovery protocols included a methodical drying process using a gentle fabric to alleviate stress and facilitate thermoregulation in the rodents after each swim session.

Voluntary running wheel

The running wheel exercise (Table 2) was performed using the protocol previously described by Pryor et al. Nine days after the EAE induction, the mice were randomly placed in special cages with 24/7 access to the running wheel; the mice in the control group were placed in a cage with a locked running wheel. The odometer was used to measure the running time and the average performance intensity in 24 hours (22).

Interferon administration

The effectiveness of IFN-beta-1 in reducing the symptoms of MS has been proven; therefore, this medicine was used in the present study to compare its effects with those of swimming exercise. Accordingly, two groups of mice received 150 IU/g of IFN-beta-1 (Cinnagen, Iran) subcutaneously. IFN-beta-1 was dissolved in saline phosphate buffer. The treatment was started after the onset of MS symptoms in the mice (i.e., 9 days after EAE induction) (19).

Table 2. Exercise protocol in swimming and running wheel groups

Variables	Swimming group	Running wheel group
Environmental compatibility	One week	One week
Familiarity with exercise	4 days	4 days
Increasing over load	2-7% of body weight on day 5	Voluntary exercise
Period	4 weeks	4 weeks
Number of sessions	5 sessions per week	Whole week
Duration	30 minutes daily	24-hour free access
Intensity	4.2% body weight	Voluntary
Number of mice	18	18

Isolation of brain tissue

After 4 weeks of EAE induction, the mice were irreversibly anesthetized by combining ketamine hydrochloride (70 mg/kg) and xylazine (5 mg/kg). Then, they were sacrificed, and their brains were removed from their skulls and kept in liquid nitrogen at -80°C until their MBP levels were measured (19).

MBP measurement

MBP concentration was measured by an enzyme-linked immunosorbent assay (ELISA) utilizing the Emax MBP ABIN368085 kit from the UK, according to the manufacturer's protocols. The process commenced with the extraction and homogenization of cerebral tissue in a solution containing 137 mM sodium chloride, 20 mM Tris-HCl at a pH of 8.0, 1% NP40, glycerol 10%, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 µg/mL aprotinin, 1 µg/mL leupeptin, and 0.5 mM sodium orthovanadate. To achieve a physiological pH of 7.5, a 1 N sodium hydroxide solution was added to the homogenates. After this adjustment, the samples underwent centrifugation at 14000 rpm for 3 minutes at 4 °C, and the supernatant was removed. The next stage included the preparation of ELISA wells, which were first coated overnight with a carbonate buffer. This was followed by a blocking step where the block buffer and the sample were introduced to the wells for 1 hour. Afterwards, samples and standards were applied to the wells and incubated for an additional two hours at ambient temperature. A standard curve was meticulously constructed using predefined MBP concentrations to facilitate the determination of MBP levels in the samples by comparison. These concentrations were measured with an ELISA plate reader and normalized against the protein content of each sample, with final values expressed in p/mL of protein.

Statistical analysis

To analyze the data and examine the research hypotheses, first, the normality of the distribution of the data and the homogeneity of the variances were evaluated using the Shapiro-Wilk test (it was more than 0.05, and the distribution was normal) and the Levine test, respectively. Then, according to the results of these two statistical tests, appropriate parametric tests were used to check the research hypotheses (one-way analysis of variance test and Tukey's post-hoc test). SPSS version 24.0 software was used to analyze the research data, and Excel 2016 software was used to draw the graphs. In this research, the significance level was $P \leq 0.05$.

Results

All data had a normal distribution. As shown in Figure 1,

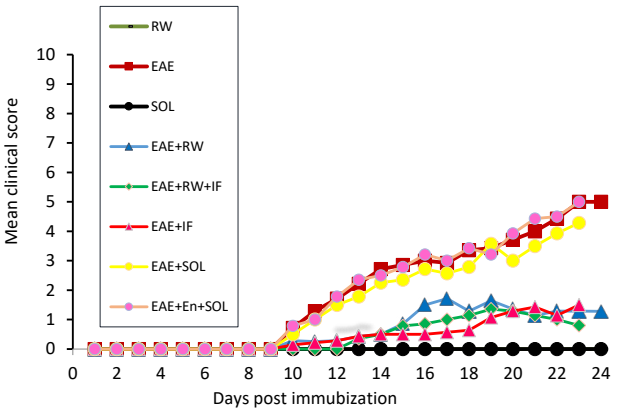

Figure 1. Clinical symptom scores in different running wheel groups ($P=0.001$): EAE (MS group), RW (running wheel group), Con (control group), EAE + IF (MS and interferon group), and EAE + EN + SOL (MS, environment, and saline injection group). No significant difference was observed between the EAE + running wheel, EAE + interferon, and EAE + running wheel + interferon groups

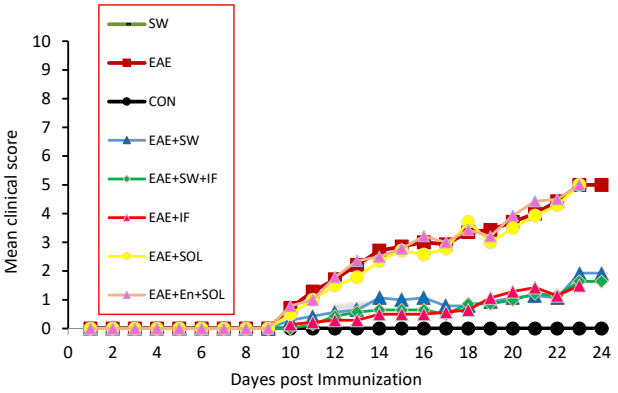

Figure 2. Clinical symptom scores in different swimming groups ($P=0.001$): EAE (MS group), SW (swimming group), Con (control group), EAE + IF (MS and interferon group), and EAE + EN + SOL (MS, swimming environment, and saline injection group). No significant difference was observed between the EAE + swimming, EAE + interferon, and EAE + swimming + interferon groups

the EAE group showed more severe clinical symptoms than the other intervention groups, indicating the successful induction process in the EAE model in the present study. The results presented in Figure 1 indicated that the running wheel exercise effectively reduced the clinical symptoms of EAE mice ($P=0.001$). Moreover, the effect of voluntary running wheel exercise on MS was almost identical to the impact of interferon on MS. Overall, no significant difference was observed between the EAE + running wheel (0.5), EAE + interferon (1.5), and EAE + running wheel + interferon groups (1.0) ($P=0.09$).

As shown in Figure 2, the EAE group showed more severe clinical symptoms than the other intervention groups, indicating the successful induction process in the EAE model in the present study. The results presented in Figure 2 indicated that swimming exercise effectively reduced the clinical symptoms of EAE mice ($P=0.001$). Moreover, the effect of swimming exercise on EAE was almost identical to the impact of interferon on EAE. Overall, no significant difference was observed between the EAE + swimming (1.9), EAE + interferon (1.5), and EAE + swimming + interferon groups (1.6) ($P=0.09$).

As seen in Figure 3, the results of ANOVA showed that EAE significantly decreased MBP in the brain tissue of mice with EAE in the running wheel groups compared to those in the control group ($P=0.001$). This significant decrease was also observed in the EAE + SOL group ($P=0.02$) and EAE + EN + SOL ($P=0.03$) groups. Further analyses indicated that exercise alone, interferon alone, or exercise in EAE mice led to higher levels of MBP compared to the EAE group; this increase in the EAE + exercise + interferon groups was statistically significant ($P=0.01$).

ANOVA results presented in Figure 4 indicated that EAE in the swimming groups led to a significant decrease in MBP level. However, the reduction was not significant ($P=0.09$). This significant decrease was also detected in the EAE + SOL ($P=0.04$) and EAE + EN + SOL ($P=0.041$) groups. Further analyses indicated that swimming alone, interferon alone, or swimming in EAE mice led to higher levels of MBP compared to the EAE group; however, this increase was not statistically significant ($P=0.07$).

Discussion

The results showed that lesion scores were lower in EAE mice performing swimming and running wheel exercises than in the control group. According to the results of this study, EAE-induced mice had higher scores than those in EAE + exercise groups, indicating that exercise and IFN-beta-1 led to more positive results in the examined time range. Overall, no significant difference was observed between the EAE + exercise, EAE + interferon, and EAE + exercise + interferon groups. However, according to the score chart, the average score of severity of injury in the EAE groups was 2-3, indicating controlled and reduced progression of demyelinated lesions as a result of exercise. Research suggests that exercise has therapeutic benefits for MS patients (23), influencing oligodendrocyte proliferation and repopulation, remyelination, neuroinflammation, and neuroprotection (23). Myelin plays an essential role in the physical and mental health of patients with MS (24). However, there needs to be more information about the effects of different types of aerobic exercise on clinical symptoms and myelin production in MS patients. The results of the spinal cord flow cytometry analysis in the study by Bernardes et al (21) showed that the volume of demyelination was significantly lower in mice in the exercise group than in the inactive group. It has been suggested that protection against myelin degradation may result from increased proliferation of oligodendrocyte progenitor cells (OPCs) and the maturation of oligodendrocytes (25). In the present study, EAE reduced MBP levels. In line with this result, Klaren et al stated that the animal model of EAE seems ideal for determining the effects of exercise on MS pathophysiology (1). Researchers have reported that EAE decreased MBP expression in the hippocampus of the C57BL/6 mice (26). Evidence suggests that 4 weeks of aerobic exercise (for

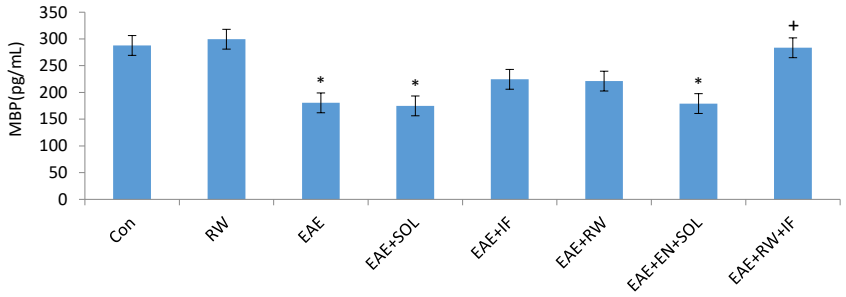

Figure 3. Comparison of the mean MBP concentration in different running wheel groups. (*significant difference compared to the healthy control group), (+significant difference compared to MS group; $P=0.01$), EAE (MS group), RW (running wheel group), Con (control group), EAE+IF (MS and interferon group), and EAE+EN+SOL (MS, running wheel environment, and saline injection group)

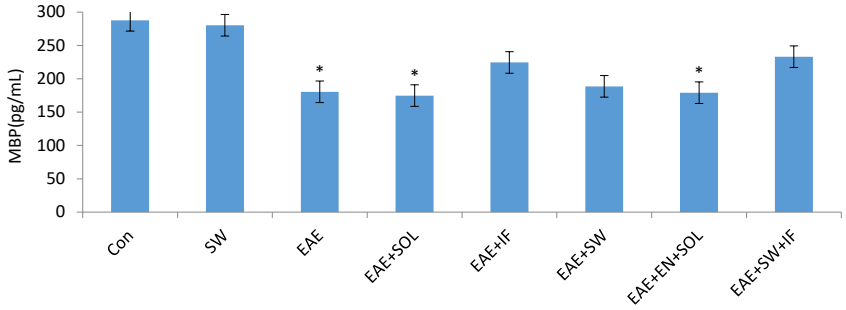

Figure 4. Comparison of the mean MBP concentration in different swimming groups. (*significant difference compared to the healthy control group), EAE (MS group), SW (swimming group), Con (control group), EAE+IF (MS and interferon group), and EAE+EN+SOL (MS, swimming environment and saline injection group)

example, swimming, walking, cycling, and running on a treadmill) improves walking performance in MS patients and increases MBP in their brains. However, this increase was not significant in the present study. Researchers have also shown that aerobic exercises are effective in improving MS patients' overall condition. However, those results were not statistically significant as well (27). The intensity and duration of training account for different results. In C57BL/6 mice with chronic EAE, a significant increase in MBP level was observed after 4 weeks of forced swimming exercise (28). Moreover, the protective effects of swimming on axonal damage in the spinal column of EAE mice were also reported (29). Recently, it has been shown that swimming inhibits inflammation and demyelination and improves motor function and clinical damage in EAR rats. According to the results of this study, swimming exercises can be performed to improve MS symptoms (8). Other studies have reported that 6 weeks of aerobic swimming before EAE induction in C57BL/6 mice increased MBP and PLP expressions in their spinal cords, which is not in line with the findings of the present study; however, these differing results might be due to the different exercise protocols used in the two studies (21,26). The study examined the effect of 4 weeks of voluntary running wheel exercise on MBP level. The results indicated a significant increase in MBP, consistent with the results of the study by Devasahayam et al. In that study, running wheel exercise significantly improved neurotrophic factors and neutrophils in muscle and nerve tissues of EAE mice (27). Patel and White examined the effect of a 10-day treadmill exercise on neurotrophic factors in EAE animals. They reported that NGF levels significantly increased in the exercise groups (30). In this regard, researchers noted that the effects of walking on a treadmill on nerve proteins in mice with cerebral palsy were associated with MBP stabilization and increased myelination (31). The levels of myelin oligodendrocyte glycoprotein and clinical disability scores of EAE mice increased after 50 days of running wheel exercise (32). A study also showed that 30 days of running wheel exercise improved the clinical scores of male EAE mice (33). This exercise is associated with enhanced myelination in the cerebral cortex of EAE mice and the acceleration of the generation of myelin and proliferation of OPCs; therefore, it can help understand the beneficial effects of exercise on CNS myelination and its underlying mechanisms (34).

Conclusion

The overall results of the present study showed that swimming exercise maintained and increased MBP levels in the brains of EAE mice. Although this finding was not statistically significant, it seems appropriate to recommend water sports to MS patients. However, the results of running wheel exercise were statistically significant; therefore, this type of aerobic exercise is more effective than swimming. Therefore, considering the effects of the kind of exercise environment on different neurotrophic factors, especially MBP level, the protocols used in this study can be applied to reduce clinical symptoms of MS and increase the amount of MBP in the brains of MS patients.

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University.

Authors' Contribution

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Competing Interests

The authors declare that there is no conflict of interests.

Ethical Approval

This study was approved by the Ethics Committee of the Institute of Physical Education and Sports Sciences (IR.SSRC.REC.1402.158).

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